PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: LeCluyse, Edward L., et al.

Group Art Unit: 1651

Examiner: Afremova, V.

Serial No.: 09/527,352

Docket No.: 421/17/2

Filed: March 17, 2000

For: METHOD OF SCREENING CANDIDATE COMPOUNDS FOR SUSCEPTIBILITY TO BILIARY EXCRETION

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DECLARATION PURSUANT TO 37 C.F.R. § 1.131

Commissioner for Patents
Washington, D.C. 20231

Sir:

1. I, Edward L. LeCluyse, am a co-inventor of the invention disclosed and claimed in the subject above captioned U.S. Patent Application Serial No. 09/527,352.

2. I have had an opportunity to review the Official Action mailed on March 13, 2001 from the U.S. Patent and Trademark Office for the above-referenced U.S. patent application.

3. I have also reviewed the following documents cited by the United States Patent and Trademark Office in the Official Action mailed on March 13, 2001:

(a) Liu et al., "Biliary Excretion In Sandwich-Cultured (SC) Hepatocytes: A Novel *In Vitro* Model System for Investigating Biliary Excretion," *Pharm. Sci.* 1:S-119 (1998). (Abstract)

4. The Invention embodied in claims 1-64 of the subject U.S. patent application was invented prior to the November 16, 1998 publication date of Liu et al. [CC].

5. Attached hereto as **Exhibit A** is a true and accurate redacted copy of an invention disclosure document submitted to the Office of Technology Development at the University of North Carolina at Chapel Hill. **Exhibit A** describes the invention embodied in claims 1-64 and predates the November 16, 1998 publication date of Liu et al. [CC].

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Edward L. LeCluyse

Edward L. LeCluyse

9-10-01

Date

EXHIBIT A

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THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

REPORT OF INVENTION

1. **DISCLOSING PARTIES*:**

1 Edward L. LeCluyse 2 Kim L.R. Brouwer 3 Xingrong Liu

	1	2
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*We ask for "disclosing parties" rather than "inventors" because an inventor is one who contributes to the conception of an invention as that invention is subsequently defined by one or more patent claims; therefore the final determination on inventorship must wait until such time as a patent application is filed.

**If any of the inventors were employed by other institutions while the invention was being made, please include the name, address and phone number of that institution.

[FOR ADDITIONAL DISCLOSING PARTIES, PLEASE USE THE SAME FORMAT AS ABOVE AND ATTACH AS AN ADDENDUM AT THE END OF THIS REPORT]

Pursuant to the Patent Policy of The University of North Carolina at Chapel Hill, I/we hereby disclose details about the following invention:

2. **TITLE OF INVENTION:**

In Vitro Hepatocyte Culture System as a Screen for Biliary Excretion

3. **DATE OF INVENTION:** [Indicate actual or approximate dates.]

Earliest conception*:
Experimentation Period:
Reduction to Practice**:

Are experimental data validating the invention or prototypes of the invention available?

YES

*Conception means the formation, in the mind of the inventor(s), of a definite and permanent idea of the complete and operative invention as claimed, as it is thereafter to be applied in practice.
**If the invention has not been reduced to practice, please so indicate.

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4. **DESCRIPTION OF INVENTION:**

a. Classify invention as one or more of the following: a new process, composition of matter, a device, or an improvement to an existing process, composition of matter or device.

A new process

b. Write a brief descriptive abstract of the invention without disclosing any confidential information. This may be used for marketing purposes.

Drugs that are destined to be extensively secreted into bile can alter the pharmacological and/or toxicological effects of other drugs or may never attain adequate therapeutic levels. In either case, early detection of such candidates during the process of drug discovery and development is becoming more and more essential in this modern era of synthetic capabilities (e.g., combinatorial chemistry approaches). Current methods of screening lead and backup compounds as substrates for biliary elimination involve *in vivo* or *in situ* treatment of animals, which is a costly and time consuming process. In addition, little is known about the species differences in the biliary excretion of drugs and their metabolites, partly due to the lack of adequate *in vitro* model systems, particularly for humans. Therefore, there is a need for an *in vitro* tool that is not only rapid and inexpensive but also predictive of hepatobiliary disposition in humans. Currently no such model system has been reported. Our methods of maintaining primary cultures of hepatocytes offer new and exciting possibilities for examining biliary transport function *in vitro*. Recent advances in cell culture technology have shown that manipulating the extracellular matrix configuration can have profound effects on their ability to form elaborate networks of functional bile canaliculi. These networks of bile channels represent separate and discrete compartments within which drugs and their metabolites are secreted as *in vivo*. We have developed methods for assessing the excretion of drugs into the canalicular compartment in monolayer cultures of hepatocytes, thus allowing us to determine a drug's potential for biliary elimination.

c. Expand on novel and unusual features which distinguish this invention from present technology. What problems does the invention solve or what advantages does it possess?

Hepatic elimination of xenobiotics involves a complex set of physiological and biochemical processes. Due to the obvious difficulties in studying these processes in humans, the rat has been the primary species in which xenobiotic biliary excretion has been examined utilizing a variety of *in vivo* and *in vitro* techniques (*e.g.*, isolated perfused livers, hepatocyte suspensions, isolated/highly purified bLPM and cLPM vesicles). While bLPM and cLPM vesicles represent an ideal model system for mechanistic studies, and have been employed in limited hepatic transport studies in humans, isolation of pure fractions, and transport studies in these vesicles, are not trivial procedures. Moreover, data generated in this system may not indicate how the intact cell or organ responds in the presence of transport, binding, and/or metabolism at other sites. On the other hand, the opportunity to extend mechanistic studies of hepatobiliary transport to humans is limited with intact organ models.

Primary cultures of hepatocytes that form intact canalicular networks sealed by tight junctions, allowing a readily accessible compartment for quantitation of substances excreted into bile, offer a distinct advantage over existing methodology. Pioneering work with hepatocyte couplets demonstrated the utility of a hepatocyte system that formed discrete bile canaliculi; however, only ~8% of cells paired to form canaliculi. Subsequently, LeCluyse *et al.* (1994) demonstrated that rat hepatocyte cultures maintained in a collagen-sandwich configuration developed a contiguous anastomosing network of bile canaliculi throughout the culture; the staining pattern for apical markers corresponded with the bile canaliculi and was distinct from basolateral membranes. In cultures maintained for 3-4 days in a sandwich configuration, the fluorescent dye carboxyfluorescein (CF) is localized almost exclusively in the bile canalicular networks, consistent with canalicular transport by the cMOAT. Treatment of hepatocyte cultures with EDTA disrupted the tight junctions sealing the canaliculi where the CF was concentrated, allowing direct access to the bile compartment. This novel *in vitro* model system offers some exciting possibilities for examining canalicular transport processes and

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mechanisms of hepatobiliary disposition, particularly, elimination of drugs. There is a growing interest in predicting xenobiotic/metabolite systemic disposition, routes of hepatic excretion, and interactions between xenobiotics within the human hepatobiliary system for pharmacological and toxicological purposes. Such a model system also will help in our understanding of the mechanisms involved in hepatic translocation of xenobiotics/metabolites in healthy and diseased subjects.

d. Comment on possible uses for the invention. In addition to immediate applications, are there other uses that might be realized in the future?

It is anticipated that there will be an immediate need for this model system to help screen new drugs for their propensity to be extensively eliminated into the bile *via* first pass metabolism. Other uses may be as a screen to test drugs as substrates or inhibitors for specific hepatobiliary transporters.